C16. **Flow cytometry support in diagnosis of hypersensitivity etiology**

Claude Lambert
Immunology Lab Univ Hospital Saint-Etienne, France

Hypersensitivity (HS) is a common, clinical event that can have life threatening issue, every year responsible for death of people at any age, most of the time healthy and with rare predictive alerts. Causes of sever HS are multiple: vegetal, food, chemicals, insect stings and most of all medicine (including antibiotics, anesthetic drugs, anti-tumor chemotherapy, steroids...). The frequency of allergy is rising every year and is much more frequent in western countries raising the "hygiene hypothesis", with several possible mechanisms.

It is nowadays generally accepted that there are different types of HS: one NON immune HS (involving specially drugs and chemical) through pharmacological activities and the other ones, highly specific immune HS that correspond to a normal sensitization but a highly exaggerated response. From the immune HS we can clearly distinguish

- immediate hypersensitivities (IHS) occurring within 20 min of exposure, that involves Immunoglobulin E, and possibly some IgG on mast cells and basophils and
- delayed type hypersensitivity (DTH) that needs at least 2 to 3 days and involve T cells with antigen presentation.

Immune HS is a 2 step process, needing first a sensitization process and then the exacerbated crisis itself. The first step is most of the time silent and not diagnosed. The HS can only be diagnosed through effector crisis. There are many symptoms associated with HS. The most frequent are asthma, eczema, urticaria, rhinitis but also anaphylactic shock, sudden death, angio-oedema, Stevens-Jonhson, Lyell, Toxic necrotic epidermolysis syndrom. HS crisis are repeated at each new contact with most of the time increasing gravity but the first reaction can already be life threatening while other HS decline may with time.

Diagnosis of allergy etiology is frequently difficult. A strict inquiry should lead to possible candidates that must be confirmed. The most robust confirmation tools are clinical provocation tests through skin tests and possible challenges. This may be dangerous and is not always possible to perform safely. Dosage of immunoglobulin specific for allergen is a precious, objective and quantitative confirmation of the sensitization but does not cover all types of HS and are not absolutely predictive of the clinical risk. The most predictive, and safe tool for allergy is then the ex-vivo provocation tests by challenging effector cells with the possible allergens and Flow cytometry is ideal for this.

Cell tests for the DTH are then T cell activation tests challenged with allergens. The reading need 2 to 7 days incubation and the read out can be cell activation (CD25, HLA-DR...), cytokine production (IFN□, IL-17...) and cell proliferation. IHS cell tests are performed on peripheral blood basophils, with immediate (10-30 min). Few procedures have been proposed to identify basophils and the read out is measuring membrane expression of internal proteins externalized during degranulation

The diagnosis role of these tests are: confirmation of HS, identification of the allergen and its component involved, identification of possible cross-reacting allergens, well tolerated molecules and possible follow up on time, especially in case of spontaneous or therapeutic decline.

The aim of this talk will be to overview the different flow cytometry tools in diagnosis of immune HS with a special focus on IHS.